that could separate them from the families linked to the chromosome 2 NPH locus (named NPH1). This reveals genetic heterogeneity also in the purely renal form of NPH. *In situ* hybridization of YAC clones isolated with 2 closely linked markers assigned the NPH1 regions to 2q13. Futhermore, based on haplotype analysis and specific recombination events, the NPH1 gene has been placed between D2S293/D2S340 and D2S121, a genetic interval of about 5 to 7 cM.

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Role of modifier genes in PKD

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We are studying a new spontaneous mutation, *nm1633*, that is an autosomal recessive located on mouse Chromosome 8. Mice homozygous for the mutated allele are runts during the suckling period and manifest an unusual facial dysmorphism at the time of weaning. After weaning, the testes fail to develop normally and undergo degenerative changes resulting in male sterility. The kidneys develop a progressive polycystic disease with a pathology remarkably similar to human autosomal dominant PKD. The *nm1633* mutation occurred on the C57BL/6J inbred strain. On this genetic background, renal cysts grow at a remarkable constant rate during the first year of life with the wet weight of the kidneys doubling every 120 days. Affected animals that survive weaning appear to die of renal failure at 8 to 12 months of age, and males live slightly longer than females.

During our initial genetic studies, we used a linkage testing stock, Mev/Ty, to determine the chromosomal location of the *nm1633* mutation. Initial difficulties occurred when we were unable to identify mutant animals in the F2 generation using the criteria of decreased body size and the presence of a readily apparent facial dysmorphism at weaning. Gross examination of the kidneys at 6 to 8 weeks of age also failed to reveal the presence of cysts. Since renal cysts were readily apparent at this age in C57BL/6J mice, we concluded that genetic background had a dramatic affect on the rate of cyst growth. We were eventually able to map the *nm1633* mutation to Chromosome 8 by waiting until the F2 animals reached 10 months of age when histological examination of the kidneys confirmed the presence of cystic disease in approximately 25% of the mice.

Next, we generated an intercross between *nm1633*-C57BL/6J and an inbred strain of Mus castaneus, CAST/Ei. Because of the relatively large genetic variation between these two species of mice, we reasoned it would be easier to fine map the *nm1633* mutation on Chromosome 8 in preparation of positional cloning of the locus. Similarly, scanning the genome for the presence of modifier genes would also be facilitated with this genetic cross. Using simple sequence length polymorphisms (SSLPs) and the (C57BL/6J × CAST/Ei)F1 intercross, *nm1633* was fine mapped on Chromosome 8 and a yeast artificial chromosome (YAC) contiguous of the region is now almost complete.

Surprisingly, the average rate of cyst growth in the CAST/Ei intercross was increased rather that decreased as observed with Mev/Ty. To map the CAST/Ei modifier gene allele responsible for the increase severity of cystic disease, we scanned the genome of 43 mutant F2 mice using 3 to 6 SSLPs per chromosome. Analysis of the log wet kidney weight at 6 months of age and the genotype of all loci examined using MAPMAKER/QTL, identified a modifier gene on distal mouse Chromosome 19 near D19Mit1 (LOD score 3.11) that was responsible for a significant amount (30% to 50%) of the variation of cystic disease observed in the F2 animals.

A second method used to attempt cloning the *nm1633* mutation and possibly identify modifier genes was differential screening of a mouse kidney cDNA library using radiolabeled cDNA probes from normal and mutant C57BL/6J mice. Two clones (nm1633A and nm1633B) were identified that showed three- to fourfold reduced expression in the mutant kidney. The nm1633B clone mapped to distal Chromosome 6 near Kap which encodes the androgen-inducible protein, KAP. DNA sequence analysis confirmed that nm1633B was KAP. Interestingly, Calvet and coworkers have reported reduced expression of KAP in mice with congenital polycystic kidney disease, *cpk*, a mutation that maps to proximal Chromosome 12.

DNA sequence analysis of the nm1633A cDNA also generated unexpected results. The DNA sequence was strongly related to the DNA sequence of a human SA protein cDNA clone. The SA protein was originally identified in the kidney of hypertensive SHR rats because expression was increased approximately tenfold over the observed in WKY normotensive rats. For this reason, SA protein gene is considered a candidate gene affecting severity of essential hypertension in rats. Our protein database searches for other amino acid sequences related to both nm1633B and the SA protein also yielded surprising results. The MACAW protein sequence alignment program showed that both genes are members of a super-gene family of enzymes that bind ATP and adenylate substrates as part of a two-step mechanism that eventually generates AMP and PPi as end products.

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Insertional mutagenesis and PKD

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The molecular charcaterization of genes associated with polycystic kidney disease (PKD) in the mouse represents an important means of establishing the molecular basis of renal cyst formation in both mice and humans. We have identified a new recessive PKD mutation in mice, called TgN737Rpw. Mutant animals in this line develop bilateral polycystic kidneys and abnormalities associated with the intrahepatic biliary duct. Gross examination of mutant kidneys revealed that they were slightly enlarged, pale and contained numerous cysts with concurrent destruction of the surrounding parenchyma. The livers were slightly pale with a prominent reticular pattern. Histologically, the lesions in the kidneys and livers of TgN737Rpw mutant mice were remarkably similar to those seen in human ARPKD. The cardinal features of both the kidney and liver lesion typical of human ARPKD were a constant finding in the mutant animals. In the kidneys, an initial mild, multifocal, microscopic dilation of the proximal tubules was followed rapidly by marked dilation and cyst formation of the collecting tubules.

The unique feature of the Tg737Rpw mutation is that it arose by insertional mutagenesis in one line of our transgenic mice. Utilizing transgene sequences as a molecular "tag" for the integration site in the Tg737Rpw mice, we were able to clone and characterize the mutant locus and identify a candidate PKD gene whose expression is directly affected by the mutation in this line. This was done initially by screening a mutant genomic library with a probe corresponding to the integrated transgene, which subsequently allowed us to clone genomic sequences flanking the transgene. Unique copy probes derived from the flanking sequences were then used to clone the corresponding wild-type sequences. Comparison of the wild-type and cloned mutant regions allowed us to derive the structure of the mutant locus. The transgene integration resulted in the deletion of about 2.5 kb of host sequences from the integration site, and the co-integration of an approximately 23 kb genomic fragment with the transgene sequences.

A gene that spans the transgene integration site was identified, which we are calling the Tg737 gene. This gene is normally expressed with a broad tissue distribution, including the adult liver and kidney, and gives rise predominantly to a 3.2 kb mRNA. Several cDNA clones representing the Tg737 gene were isolated and sequenced; one of these, cDNA5, was determined to be 3045 bp in length, and to contain a long open reading frame that potentially represents the entire peptide encoded by the predominant 3.2 kb mRNA species. Analysis of mutant tissues revealed that the mutation in this line prevents expression of the 3.2 kb mRNA.

The protein predicted by cDNA5 is unique and has not been described previously in GenBank. Analysis of the primary amino acid sequence and a search with the BLAST network revealed that the protein contains ten copies of an internally repeated degenerate 34-amino acid sequence referred to as the tetratricopeptide repeat (TPR). The TPR was first described in lower eukaryotes as a motif associated with several genes involved in cell cycle control. More recently, TPR-containing proteins in yeast and Drosophila have been discovered that function in various cellular processes including protein import, transcription and neurogenesis. Although it is presently unclear precisely how the normal expression of the Tg737 gene might prevent PKD in mice, because TPR genes are associated in some cases with cell cycle control, we are hypothesizing that this gene may function by controlling epithelial cell proliferation and/or by regulating the apoptosis that normally participate in kidney development.

Additionally, utilizing the mouse cDNA as a probe, we have cloned and sequenced the homologous human gene and have determined its intron/exon structure. Based on this analysis, we have determined that the human gene is greater than 100 kb in size, and contains 26 exons, most of which are less than 100 bp in length. The human mRNA is smaller than the mouse, due primarily to a deletion of approximately 293 bases from the 3′ UTR. Comparison of the ORF within the human and mouse cDNA clones revealed that both contain the same number of TPR motifs, and both have the potential to encode proteins that are highly homologous and essentially the same size. The molecular reagents derived from the analysis of the TgN737Rpw mutant are allowing us to analyze DNA from human families with PKD in order to determine whether the Tg737 gene is associated with any genetic disease conditions in humans that involve the development of renal cysts.

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